Measuring Compartment Size and Gas Solubility in Marine Mammals

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LONG-TERM GOALS

The long term goal of this study is to develop methods to estimate marine mamal tissue compartment sizes, and tissue gas solubility. We aim to improve the data available for the relative size of different tissues in various marine mammal species, as well as our understanding of their different morphological and physiological adaptations. The study will also develop a method that enables the determination of the gas solubility in different tissue compartments.

OBJECTIVES

This study include two main objectives: to study the morphometrics of marine mammal compartments and the solubility coefficient of these compartments. Both objectives need the development of new methods to reach their respective goals.

The first objective is aimed at improving the data available for the relative size of different tissues in various marine mammal species, as well as our understanding of the different morphological and physiological adaptations that exist among marine mammals. Previous efforts have been focused on measuring the major O_2 stores, such as muscle mass and myoglobin (Mb) concentration, or total blood volume and hemoglobin content [1]. There is also little or no information for certain tissue compartments such as skin, blubber, muscle, heart, lung, liver, kidneys, spleen or bone. The relative size of each compartment has not been properly calculated with a consistent methodology.

Therefore there is a need to consistently measure the relative size of the different tissues: such as skin, muscle, blubber, heart, and lungs in as many species as possible.

The second objective is aimed at developing a method that enables the determination of the gas solubility in different compartments. There are limited data on gas solubility in marine mammal tissues: species differences have been found and variations compared to land mammals are expected [2]. We aimed to modify methods published in the human literature to study gas solubility of anaeshtetics in human and animal tissue [3-5] to determine nitrogen solubility in tissues of marine mammals

APPROACH

OBJECTIVE 1
For this aim fresh specimens of adult animals will be requested from different locations: North Carolina, Cape Cod Bay and from the Canary Islands. In addition, access to bycaught animals will be facilitated by NOAA. A mass dissection protocol to systematically separate the body into discrete anatomical components will be developed in collaboration with McLellan and Pabst, based on their previous experience [6]. Tissues will be weighed separately. Volume will be measured by water displacement. Density will be calculated by dividing the weight by the volume. Finally, we will report the mass of each body compartment as a percentage of the total body mass in accordance to Grand [7].
Aim 2: Muscle myoglobin determination. Myoglobin content will be calculated for the different muscle groups, including heart, of each specimen following the method described by Polasek and Davis [8].

OBJECTIVE 2
We will follow methods published in the human literature to study gas solubility of anaeshtetics in human and animal tissue [3-5], but modified so we work under a control environment free of nitrogen. This method was developed initially at WHOI and during this year it has been transferred to the canaries (at the ULPGC) with the aim of getting samples from deep divers.

WORK COMPLETED

OBJECTIVE 1
Aim 1: Obtaining morphometric data of different species.
Mass data of 21 marine mammals belonging to 11 species have been collected. The high variety of species was reached due to the collaboration of multiple stranding networks along the US Coast (IFAW, NCW, TMMC), and the Canary Islands (ULPGC), as well as the North East Fisheries Marine Mammal Observers Program. The targets of the study were fresh (code 2) adult animals in good body condition. However mass dissection data were taken from animals with different body condition given the small sample size. As the sample size will increase, the more skinny animals will be excluded from this study. Meanwhile the mass dissection data from the animals with poor body condition contribute to our understanding of the health status of these animals. Early code 3 animals from species of interest were also included. Only one code 4 was studied (*Grampus griseus*). The bone weight was the only weight studied since it was the only body compartment that might be still unaltered by decomposition. In two animals (*Phocoena phocoena* and *Kogia breviceps*) total weight was not measured but was estimated summing all the weighted tissues plus the mean value of non-study tissues of animals of similar size (Table-1).

Table-1: List of studied animals including specie, ID number, stranding coast and other relevant biological data.

Specie	ID	Stranding coast	Age	Sex	Body condition	Decompositon code	Weigh (g)	Lenght (cm)
P. phocoena	IFAW14-034Pp	Cape Cod	subadult	Female	skinny	2	-	120
D. delphis	IFAW14-044Dd	Cape Cod	Subadult	Male	Robust	2	68800	176
D. delphis	IFAW14-116Dd	Cape Cod	Adult	Male	Fair/Good	2	94300	221
D. delphis	IFAW14-134Dd	Cape Cod	Subadult	Male	Robust	2	134000	221
S. frontalis	WAM689	Wilmington	Subadult	Female	Good	2	67000	180.5
T. truncatus	WAM690	Wilmington	Subadult	Male	Fair	2	89000	195
M. angustirostris	ES3607 (Lucky duck)	California	Calve	Male	Cachexic	2	33500	121
Z. californianus	CSL10941 (Mohawk)	California	Adult	Male	skinny	2	111500	197
Z. californianus	CSL10972	California	Adult	Female	skinny	2	62500	151
Z. californianus	CSL10466	California	Adult	Female	skinny	2	71500	160.5
H. grypus	DO9587	Cape Cod	Subadult	Female	Robust	2	36000	104
H. grypus	IFAW14-114Hg	Cape Cod	Juvenile	Female	Robust	2	25000	99
S. coeruloalba	CET732	Canary Islands	Juvenile	Female	Fair	2	37000	151
G. macrorhynchus	CET739	Canary Islands	Juvenile	Female	Fair	3	119000	202
D. delphis	CET745	Canary Islands	Adult	Female	Poor	2	58000	188
S. coeruloalba	CET748	Canary Islands	Adult	Male	Good	2	74300	195
S. coeruloalba	CET750	Canary Islands	Adult	Female	Poor	3	87840	214
G. griseus	CET 751	Canary Islands	Adult	Female	Good	4	242000	285.4
G. macrorhynchus	CET 758	Canary Islands	Calve	Male	Fair	3	74850	168
D. delphis	CET 767	Canary Islands	Adult	Female	Skinny	2	78900	220
K. breviceps	CET 774	Canary Islands	Adult	Male	Fair	2	248760	250

Aim 2: Muscle myoglobin determination.
Muscle samples were collected from 2 pinnipeds: *Zalophus californianus* (California sea lion) (n=1) and *Halichoerus grypus* (grey seal) (n=1), and 11 cetaceans: *Phocoena phocoena* (harbor porpoise) (n=1), *Delphinus delphis* (common dolphin) (n=4), *Stenella coeruleoalba* (striped dolphin) (n=4), *Globicephala macrorhynchus* (short-finned pilot whale) (n=1) and *Stenella frontalis* (Atlantic spotted dolphin) (n=1) in several muscles (mastohumeralis, dorsal scalenus, sternohyoid, epaxial, hypaxial and rectus abdominis), representative of different functional groups (main locomotor muscles, appendicular movement, respiration, and ingestion). Samples were wrapped in plastic wrap, placed in Ziploc bags and frozen at -20°C until analyzed. Mb concentration (g Mb100 g^{-1} wet muscle mass) of each of the muscle samples was determined using the method from Reynafarje (1963) [9]. This aim constituted a master thesis project.

OBJECTIVE 2
To determine gas solubility in tissues of marine mammals we have adapted methods published in the human literature to study gas solubility of anaeshtetics in human and animal tissue [3-5]. Major modifications are the use of evacuated tubes to help the gas to come out of solution and to work in a glove box filled with argon to minize nitrogen contamination.

Tissues have been collected for future determination of gas solubility in these tissues once the method has been developed and validated. Tissue from brain, heart, muscle, blubber, skin, spleen, kidney, liver, and lung are cut into small pieces, and immersed in saline solution before freezing of the

samples. We have tested our method using olive oil in order to validate the method. In the last year we have transferred this method to the Canary Islands. A new and improved setup is being installed there.

RESULTS

During the last year the most sucessful results were achieved with morphometric data.

OBJECTIVE 1
Aim 1: Obtaining morphometric data of different species. We have considerably increased the number of animals per species as well as the number of species studied. However, the data should still be considered preliminary since for some species the number of animals studied is still very low (1 or 2), some were calves, or in poor body condition. The size compartment of bones seems to be slightly larger in pinnipeds than in cetaceans. The size compartments of fat is larger in phocids than in cetaceans, and it is larger in cetaceans than in otariids. On the other hand the muscle compartment is similar in cetaceans and in otariids but smaller in phocids (Fig. 1).

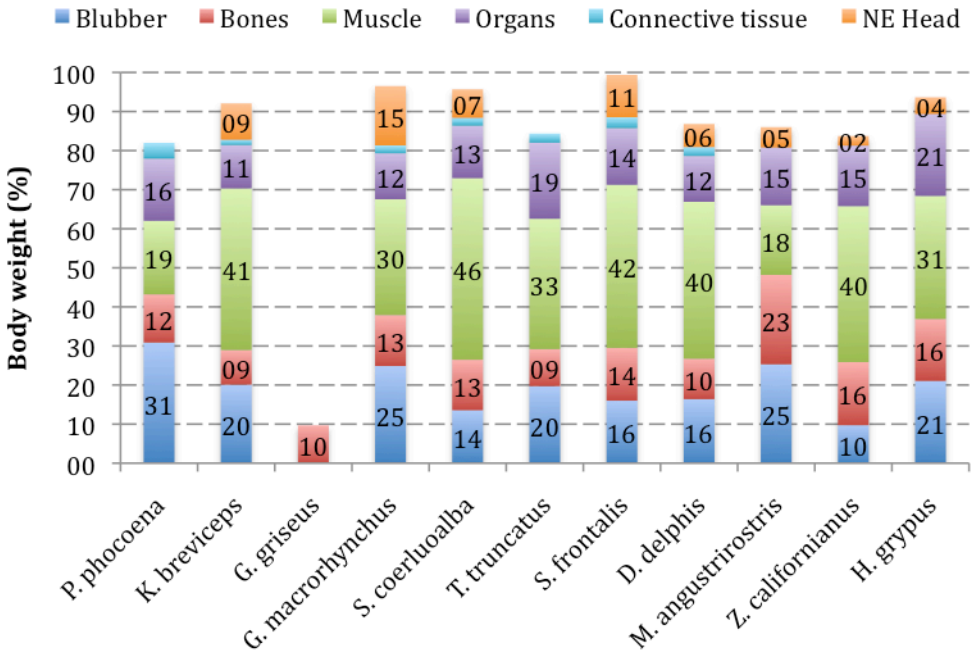

Fig. 1: Relative weight of integument (blubber), bones, muscle, internal organs, connective tissue, and the soft tissue of the head (that was calculated by substracting the weight of the skull, hyoid and brain from the total weight of the head).

Since in this study we have animals of different body condition, we have analyzed how the relative weight of the different tissues changes with total body weight. For this purpose we have focused on the common dolphin since it is the species represented with the most animals of similar age and different body conditions included in our study. Preliminary results showed that the muscle is the tissue that changed the most with body weight in common dolphins (Fig. 2). There was a very strong positive correlation between muscle mass and total body weight (r=0.96) but there was no correlation between muscle mass and total body length (r=0.28), suggesting that variation observed in muscle mass was mainly due to body condition (body weight lost) and not that much due to ontongenetic development

of the muscle. Therefore we can conclude that mass disection is an additional tool to estimate body condition and health status of animals of the same species and similar age.

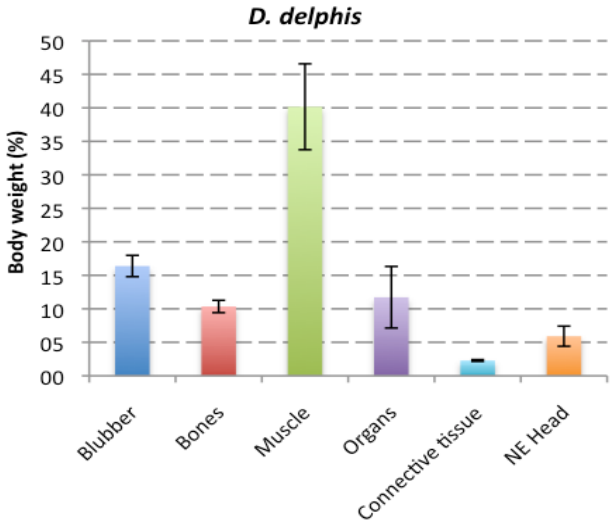

Fig.2: Mean relative body weight of integument, bones, muscle, organs, connective tissue and the tissues of the head (by inference) for common dolphins (n=5).

Fahlman et al. (2009), Hooker et al. (2009), and Kvadsheim et al. (2012) used a five-compartment model arranging the tissues in the following compartments [10-12]: blood, brain, fat, muscle (muscle, skin, bone, connective tissue and rest of organs), and central circulatory (heart, kidney, liver, and alimentary tract). Given the difficulties to estimate blood volume or blood weight from carcasses, the blood compartment was not measured in our study. The results of the five compartment model classified following Fahlman et al. (2009), Hooker et al. (2009), and Kvadsheim et al. (2012) are shown in figure 3.

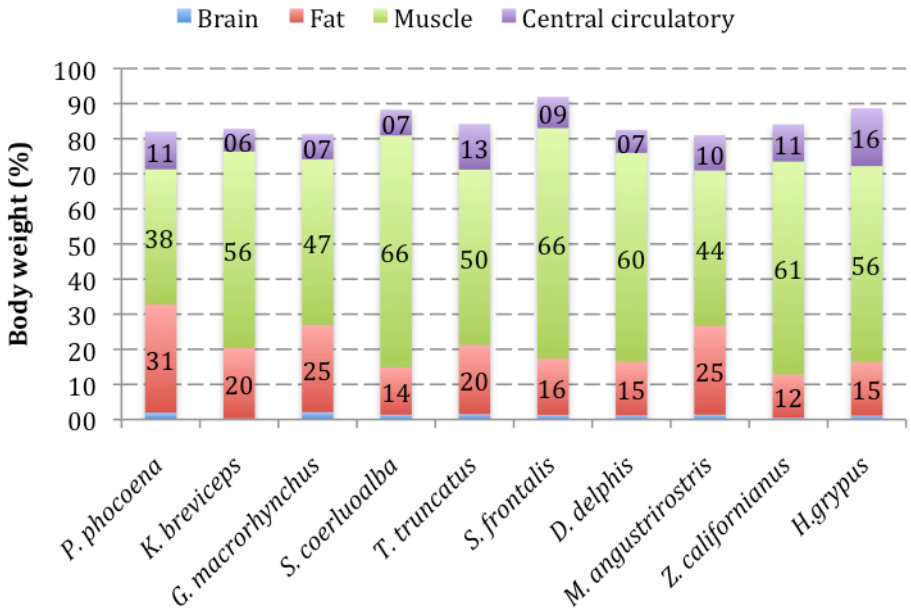

Fig.3: Mean value for the relative body weight of the compartments arranged following Fahlman et al. (2009) for each species.

During the no-cost extension of this project we plan on increasing the number animals studied for each species, the number of species, and propose a new compartment model that will reflect better the differences in body composition between species.

Aim 2: Muscle myoglobin determination.
This study has resulted in a Masters thesis project graded 8.9/10. Results showed that Mb concentration was heterogeneously distributed between and within muscles. Statistically significant differences in Mb concentration were found between locomotor and non-locomotor muscles of the species studied. Locomotor muscles were the major contributors to total muscle oxygen stores due to both high Mb concentration and a large muscle mass.

There was not one location with Mb concentration close to the mean value of the animal but mean Mb concentration values between the epaxial axilla and *rectus abdominis* locations came very close to mean Mb concentration of the animal in four out of the six adult cetaceans. Further research is needed to increase the sample size and be able to propose a sampling location representative for the animal's Mb concentration more conclusively. This location will be used to study the nitrogen solubility in muscle as nitrogen solubility might be affected by Mb concentration.

OBJECTIVE 2
Aim 2: Determine the tissue solubility coefficient of nitrogen. The previous year at WHOI we did four assays with olive oil to validate the methodology. We were able to obtain results within the numbers published in the literature, but we weren't been able to reproduce the assays. During the last year we have transferred the methodology to the Canary Islands where we have better access to deep diving animals. The transfer has not been as straight forward as planned given the different size systems used between the US (imperial system) and Europe (metric system), but at the end we hope to come up with an improved set up. We are just finishing with the installation of all the equipment, thus a non-cost extension has been applied in order to keep working on the methods and results from this objective. First we will reproduce the olive oil experiment to validate the method, then we will do the same with saline solution, and finally we will start working with the tissues of marine mammals.

IMPACT/APPLICATIONS

Prior work has suggested that marine mammals are commonly supersaturated with gas, such that a direct ascent to the surface results in bubble formation in most tissues [13]. Recent work by Bernaldo de Quiros et al [14] has shown that gas composition analysis can discriminate between gas from decompression as opposed to decomposition. Fresh, drowned-at-depth ascended bycatch do indeed show evidence of postmortem decompression from a supersaturated state [15]. How do marine mammals normally avoid DCS symptoms when at the surface? This proposal will help improve the parameters used for modeling gas management in marine mammals and improve understanding of how these animals manage gases while diving and breathing at the surface. A better understanding of their normal physiology is required to answer this question and will help determine how they normally avoid DCS. Additionally, the mass dissection has been proven to be a valid tool to evaluate body condition and health status of the animals.

RELATED PROJECTS

This project is related to N000141210388 'Markers of decompression stress of mass stranded/live caught and released vs. single stranded marine mammals' where we are using a biomarker to examine bubble stress on neutrophils and endothelial cells in diving marine mammals, in collaboration with Dr Stephen Thom at the University of Maryland.

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